



(51) International Patent Classification:

G16B 20/20 (2019.01) G16B 20/00 (2019.01)
G16B 40/20 (2019.01) G16B 30/10 (2019.01)

(21) International Application Number:

PCT/US2023/065473

(22) International Filing Date:

06 April 2023 (06.04.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/327,966 06 April 2022 (06.04.2022) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: TRUE VARIANT IDENTIFICATION VIA MULTIANALYTE AND MULTISAMPLE CORRELATION

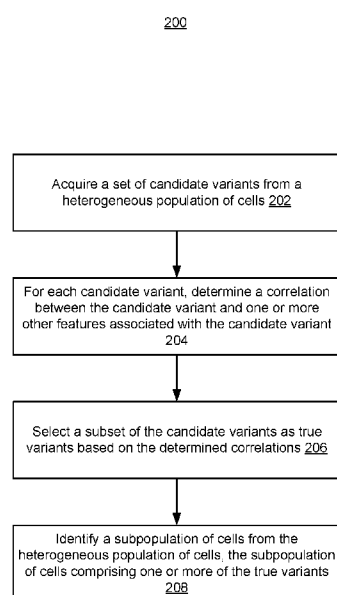


FIG. 2

(57) Abstract: Described herein are true variant identification methods via multianalyte and multisample correlations. Generally, the first step of true variant identification involves applying a first machine learned model to identify one or more correlations of a candidate variant with other analytes or variants within the same or different cells or samples. The second step of true variant identification involves applying a second machine learned model to classify a variant to be a true variant or false positive based on the identified correlations. Such improved true variant identification methods facilitate the identification of signatures of measurable residual diseases at a more granular and accurate level.

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:

07 December 2023 (07.12.2023)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/65473

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G16B 20/20, G16B 40/20 (2023.01)

ADD. G16B 20/00, G16B 30/10 (2023.01)

CPC - INV. G16B 20/20, G16B 40/20

ADD. G16B 20/00, G16B 30/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2021/067721 A1 (Mission Bio, Inc.) 08 April 2021 (08.04.2021) entire document (especially para [0014]-[0015], [0043]-[0044], [0050]-[0051], [0075]-[0076], [00124]-[00125])	1-5, 74, 77
Y	US 2020/0017917 A1 (YALE UNIVERSITY) 16 January 2020 (16.01.2020) entire document (especially para [0158], [0178], [0183]-[0188])	1-5, 74, 77

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 August 2023

Date of mailing of the international search report

OCT 23 2023

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/65473

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-27, 32-61, 65-73
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-see extra sheet-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 74 and 77

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III: Lack of Unity

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: claims 1-5, 74, and 77 are directed to obtaining a set of candidate variants determined through a single-cell analysis workflow; determining a quantity of cooccurrence cells; and identifying a subset of candidate variants as true variants based on the determined set of variant pairs.

Group II: claims 28-31, 75, and 78 are directed to generating a statistical significance by using a Beta-Binomial distribution with parameters generated from a set of control samples for a variant included in the set of obtained candidate variants; identifying a subset of candidate variants as true variants based on generated statistical significances for the variants.

Group III: claims 62-64, 76, and 79 are directed to obtaining a set of candidate variants; determining allele frequencies, wherein the allele frequencies are determined through single-cell DNA sequencing of the heterogeneous population of cells; correlating determined allele frequencies to protein expression of the proteins; a subset of candidate variants are selected as true variants based on correlation of the allele frequencies and protein expression of the proteins.

The inventions listed as Groups I through III do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2 they lack the same or corresponding technical features for the following reasons:

Special Technical Features:

The special technical feature of Group I is obtaining a set of candidate variants determined through a single-cell analysis workflow; determining a quantity of cooccurrence cells; and identifying a subset of candidate variants as true variants based on the determined set of variant pairs, not present in any other group.

The special technical feature of Group II is generating a statistical significance by using a Beta-Binomial distribution with parameters generated from a set of control samples for a variant included in the set of obtained candidate variants; identifying a subset of candidate variants as true variants based on generated statistical significances for the variants, not present in any other group.

The special technical feature of Group III is obtaining a set of candidate variants; determining allele frequencies, wherein the allele frequencies are determined through single-cell DNA sequencing of the heterogeneous population of cells; correlating determined allele frequencies to protein expression of the proteins; a subset of candidate variants are selected as true variants based on correlation of the allele frequencies and protein expression of the proteins not present in any other group.

Common Technical Feature:

Groups I through III share the technical features of a method/system for identifying a subpopulation of cells from a heterogeneous population of cells, (A non-transitory computer readable medium for calling one or more variants of a cell population, the non-transitory computer readable medium comprising instructions that, when executed by a processor, cause the processor to:) the method/system comprising:

obtaining a set of candidate variants determined through a single-cell analysis workflow; for a variant included in the set of candidate variants; determining a set of variants; identifying a subset of candidate variants as true variants; a single-cell analysis workflow device configured to generate a plurality of sequence reads for cells in a cell population; and a computational device communicatively coupled to the single-cell analysis workflow device, the computational device configured to: . However, these shared technical features do not represent a contribution over WO 2021/067721 A1 (Mission Bio, Inc.) (hereinafter Mission) 08 April 2021 (08.04.2021).

Mission teaches a method/system for identifying a subpopulation of cells from a heterogeneous population of cells (e.g. four different cell populations, para [0033]; e.g. analyzes the cell populations feature and outputs a prediction for the candidate variant, para [0077]-[0078]; e.g. heterogeneous variant, para [0085]; para [0014]-[0015], [0050]-[0051]) (A non-transitory computer readable medium for calling one or more variants of a cell population, the non-transitory computer readable medium comprising instructions that, when executed by a processor, cause the processor to (para [00124]-[00125]):), the method/system comprising: obtaining a set of candidate variants determined through a single-cell analysis workflow (e.g. the variant caller model receives the cell population features as input and predicts a classification for a candidate variant. In one embodiment, the variant caller model extracts cell population features from sequence reads that were previously corrected and predicts a classification for a candidate variant based on the extracted cell population features. [0042] The phrase "cell population features" refers to features relevant to a candidate variant derived from corrected sequence reads across a cell population, para [0042]; e.g. the cell analysis workflow device 120 can perform single-cell processing by encapsulating individual cells into emulsions, lysing cells within emulsions, para [0047]); for a variant included in the set of candidate variants (e.g. the cell population module 230 determines cell population features for each of one or more candidate variants, para [0075]-[0076]); determining a set of variants (e.g. . For additional candidate variants, the cell population module 230 determines the percentage of cells that have heterozygous copies of each of the additional candidate variants, para [0075]-[0076]; para [0073]); identifying a subset of candidate variants as true variants (e.g. a variant caller model is implemented to determine whether the candidate variant is a true variant, such as a homozygous variant or a heterozygous variant, para [0043]-[0044]);

-see extra sheet-

-see preceding sheet-

a single-cell analysis workflow device configured to generate a plurality of sequence reads for cells in a cell population (e.g. a single-cell analysis workflow device configured to generate a plurality of sequence reads for cells in a cell population; a computational device communicatively coupled to the single-cell analysis workflow device, the computational device configured to: obtain a plurality of sequence reads from cells of the cell population; for a plurality of cells in the cell population, para [0014]); and a computational device communicatively coupled to the single-cell analysis workflow device, the computational device configured to (para [0014]).

Thus, unity of invention is lacking under PCT Rule 13.1 because Groups I through III do not share a same or corresponding special technical feature that would provide a unifying contribution over the prior art. None of these special technical features are common to the other groups.